**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| Applicant | : | Judith Boston |
| App. No | : | 10/574,526 |
| Filed | : | January 23, 2007 |
| For | : | METHODS, COMPOSITIONS, APPARATUSES CONTAINING TETRAMERIC OXYGEN |
| Examiner | : | Choi, Frank I. |
| Art Unit | : | 1616 |
| Conf No. | : | 6984 |

**Mail Stop Amendment**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**DECLARATION OF JUDITH BOSTON UNDER 37 C.F.R. § 1.132**

Sir:

I, Judith Boston, do hereby declare and state that:

1. I am the inventor of the subject matter described and claimed in the above captioned patent application, and am familiar with the contents of the patent application.

2. I received an M.D. from George Washington University School of Medicine in Washington, D.C. I have been working in the field of ophthalmology for more than eight years. My *curriculum vitae* is attached as Exhibit A.

3. I am familiar with the above-captioned application as well as the Office Action dated February 10, 2009.

4. I understand that the Examiner rejected the claims, in part, for allegedly being non-enabling under 35 U.S.C. § 112, first paragraph.

5. I designed the following experiments, and they were carried at my direction with the assistance of others.

6. In a first set of experiments, a hyperbaric oxygen solution (Sante Oxygen), herein referred to as primary oxygenating ingredient (POI)) was tested for its ability to protect cells from hypoxic conditions. This is the same oxygen solution set forth in the specification of the present patent application. Human retinal pigment epithelial cells (ARPE-19 cells) were treated with POI and then placed into a hypoxic chamber for 24 hours. Control cells were incubated in the hypoxic chamber without the POI. The cells were then analyzed by phase contrast microscopy. Phase contrast images showed that the hypoxic ARPE-19 cells rounded up and displayed an unusual morphology compared to the POI treated hypoxic cells. In particular, there were 71 rounded (damaged) cells per high power field in the hypoxia treated cultures versus 8 rounded cells per high power field in the hypoxia + POI treated cultures.

7. The results of the experiments discussed in ¶6 demonstrate that the POI protected the ARPE-19 cells from being damaged by hypoxic conditions.

8. In a second set of experiments, POI was further tested for its ability to protect cells from hypoxic conditions. ARPE-19 cells were exposed for 48 hours to either: 1) normoxic conditions (Exhibit B, Untreated Normoxia) in the absence of POI; 2) hypoxic conditions in the absence of POI (Exhibit B, Untreated Hypoxia); or 3) hypoxic conditions in the presence of media containing 17.5% POI (Exhibit B, 17.5% POI + Hypoxia). Subsequently, levels of vascular endothelial growth factor (VEGF), a protein that can stimulate the growth of new blood vessels (neovascularization), were measured. Neovascularization can be induced by hypoxia and occurs in many disorders, such as ophthalmic retinopathies. (See, *e.g.*, Specification at paragraph [0027]). VEGF levels were analyzed by Western blot analyses as follows. Proteins were extracted from the cell cultures and the protein concentrations were measured. Equal amounts of protein were electrophoresed on 4-20% Tris-glycine sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gels. Proteins were then transferred to a polyvinylidene difluoride membrane. The blots were blocked for 2 hours at room temperature with 5% bovine serum albumin, Tris-saline and 0.5% Tween 20. Specific VEGF antibodies (1 μg/ml) were applied and incubated overnight at 4°C. Blots were washed with Tween-Tris-buffered saline (TTBS) and incubated for 1 hour with alkaline phosphatase-conjugated goat anti-mouse IgG antibody (1:5000). Blots were developed with a chemiluminescent substrate solution. The densities of the Coomassie blue stained SDS-PAGE gel and the VEGF Western blots were scanned in order to standardize the levels of VEGF to total protein. Data were analyzed using analysis of variance (ANOVA). As shown in Exhibit B, treatment of cells grown under hypoxic conditions with POI (Exhibit B, 17.5% POI + Hypoxia) resulted in a statistically significant reduction in the cellular levels of expressed VEGF ($p \leq 0.05$) when compared to cells grown under hypoxic conditions in the absence of POI (Exhibit B, Untreated Hypoxia).

9. The results of the experiments discussed in ¶8 demonstrate that VEGF expression increases in ARPE-19 cells under hypoxic conditions and that POI normalizes

hypoxia-induced increases in VEGF expression after a 48 hour-incubation period. Similar results were obtained when the incubation period was 6, 12, 24 or 36 hours.

10. In a third set of experiments, POI was tested for its ability to protect visual function in ischemic rabbit eyes. A component of visual function, as indicated by scotopic B wave amplitude ($\mu V$), was measured after treatment with POI in ischemic rabbit eyes. Retinal ischemia was induced in the eyes of live rabbits by intraocular infusion to create increased intraocular pressure. After induction of ischemia, scotopic B wave was markedly diminished (Exhibit C). The diminished effect was observed at 20, 40, and 60 minutes after ischemia induction (Exhibit C). After 60 minutes, POI was injected into the vitreous. After 24 hours, visual function was measured again. Visual function after treatment with POI returned to nearly baseline levels after 24 hours (Exhibit C). In contrast, in separate experiments, scotopic B wave visual function returned to less than 40% of baseline levels one week after ischemia induction without POI treatment.

11. The results of the experiments discussed in ¶10 illustrate that POI treatment demonstrates a protective effect on rabbit eyes exposed to ischemia.

12. I further declare that all statements made herein of knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

8/10/17
Date

Judith Boston, M.D.

**EXHIBIT A**

JUDITH BOSTON, M.D.

**Medical Education & Training**

Doctorate of Medicine, 1995
**George Washington University School of Medicine**
Washington DC.

Internship, General Surgery, 1995-1996
**University of California San Francisco School of Medicine**
San Francisco, California.

Ophthalmology Residency, 1996-1999
**Charles R. Drew School of Medicine**
Los Angeles, California.

Refractive Surgery Fellowship, 1999-2000
**Sinskey Eye Institute**
Santa Monica, California.

**Awards, Recognition, Honor**

Selected as one of America's Top Ophthalmologists for 2004-2005,
Consumer Research Council of America
Bristol Myers Squibb pharmaceutical company, Academic Award Recipient
American Association of University Women, Research Fellowship Award

**Professional Experience**

**Sante' jeunesse, Inc., Newport Beach CA**
President, Chief Scientific Officer- 2004-Current
Research & development of pharmaceutical products. Several patents filed.

**New Vision Medical Group Inc.** 2001-2009
Refractive Surgeon, Medical Consultant.

**Laser Eye Centers,** Los Angeles California, 2000-2001
Refractive Surgeon
All aspects of refractive surgery including refractive surgery and pre and post-operative management.

**EXHIBIT B**

Retinal Pigment Epithelial Cell VEGF Expression in Normoxia, Untreated Hypoxia and Hypoxia with POI Treatment

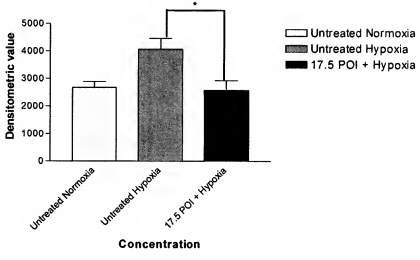

**EXHIBIT C**

Scotopic B Wave Response after Ischemia in Rabbit Eye after POI Treatment

| | | | Duration of Ischemia | | | Final Measurement |
|---|---|---|---|---|---|---|
| | Time | Baseline | 20 minutes | 40 minutes | 60 minutes | 24 hours after ischemia |
| Rabbit | POI Treatment given after 1 hour of ischemia - | 136.9 μV | 18.52 μV | 7.8 μV (no POI) | 8.85 μV (no POI) | 133.4 μV (+POI) |

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